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into the central carbonyl channel of the G-tetrad and were observed to form strong electrostatic interactions with guanines. For comparison, we studied the interaction of telomestatin with the parallel-stranded G-quadruplex and found that the G-quadruplex interaction of telomestatin is more selective for the intramolecular structure. Furthermore, the molecular dynamics docking simulations are in agreement with the experimental observations and may facilitate in the rational design of new G-quadruplex-interactive agents.

#3663 The molecular structure of G-quadruplexes from human telomeric DNA repeats: Implications for the design of drugs that interfere with telomere maintenance. Gary Parkinson, Michael Lee, Gianni I. Chessar, Martin Read, and Stephen Neidle. *CRC Biomolecular Structure Unit, Chester Beatty Laboratories, The Institute of Cancer Research, London, UK.*

The telomerase enzyme system protects telomeric ends from attrition and damage, and plays a major role in maintaining cellular immortality. We have designed a number of small molecules that inhibit telomerase by stabilising the terminal 3' end of telomeric DNA as four-stranded DNA quadruplex structures. We have determined the crystal structures of two quadruplexes formed from telomeric repeats. One structure of is an intermolecular quadruplex from two separate 12-mer DNA strands, each containing two telomeric repeats. The second structure is formed from a single strand consisting of four telomeric repeats, in all 22 nucleotides long. Both of these quadruplexes have radically different structures compared to the prevailing assumptions, with all strands having a parallel orientation and the TTA loops being embedded within the grooves rather than at the top and bottom of the stack of G-quartets. It is notable that these features are common to both structures, giving added credibility to their existence. The grooves in the structures are best described as V-shaped indentations, with complex surfaces and patterns of charge and hydrogen bonding functionality. The overall appearance of these quadruplexes resembles a disc, with the loops swung out propeller-fashion. The structures provide insight into the folding and unfolding of telomeric DNA and for ligand binding. They also indicate new opportunities for the rational design of highly specific ligands that can exploit their unique structural characteristics and discriminate against other forms of DNA structure. Such molecules may be potent effectors of telomere maintenance destabilisation in tumor cells, regardless of whether maintenance is through telomerase or ALT pathways.

#3664 Design and studies of anti-angiogenic thalidomide analogs. Pui-Kai Li, Jiandong Shi, Min Wu, Megan Marks, Zhigen Hu, Michael Ihnat, and Chandrashkhar Kamat. *College of Pharmacy, the Ohio State University, Columbus, OH, BD Biosciences, Bedford, MA, Duquesne University, Mylan School of Pharmacy, Pittsburgh, PA, and the University of Oklahoma School of Medicine, Oklahoma City, OK.*

Angiogenesis has recently become a primary target of anticancer therapy. It has been shown that thalidomide inhibits angiogenesis. Since the anti-angiogenic activity of thalidomide was reported recently, only limited structure-activity-relationship studies have been described. We first synthesized several hydroxy metabolites of thalidomide and found that 5-hydroxy (but not 4-hydroxy) and 1'-hydroxythalidomides exhibited higher anti-angiogenic activity than thalidomide. Substituting the glutaramide ring of thalidomide with an aromatic group leads to active analogs. Specifically, replacing the glutaramide ring with substituted aniline (2,6-diisopropylaniline) yielded an extremely active analog (2,6-diisopropylphenyl)-1H-isoindole-1,3-dione. We then synthesized eleven analogs of (2,6-diisopropylphenyl)-1H-isoindole-1,3-dione substituted at the 4 or 5 position of phthalimide ring to elucidate the importance of the substitutions in regard to anti-angiogenic activities. The anti-angiogenic activities of the compounds were first determined using an *in vitro* fluorescein-based cell migration assay. At 10 micromolar inhibitor concentration, thalidomide exhibited weak (21.9%) inhibitory activity of endothelial cell migration. On the contrary, (2,6-diisopropylphenyl)-1H-isoindole-1,3-dione showed 75.9% migration inhibition at the same concentration. Substitution at the 4 position of (2,6-diisopropylphenyl)-1H-isoindole-1,3-dione resulted in significant decrease (0 % - 30.4 %) in endothelial cell migration inhibition. Interestingly, substitution at the 5 position of (2,6-diisopropylphenyl)-1H-isoindole-1,3-dione obtained analogs with much higher endothelial cells migration inhibitory activities (48.9 % - 90.6 %) irrespective of the nature of the substituents. Next, the anti-angiogenic activities of the compounds were tested using the *in vivo* chicken chorioallantoic membrane (CAM) assay. We observed a direct correlation on the results obtained between the cell migration and the CAM assays. In conclusion, potent analogs of thalidomide can be obtained with modifications on phthalimide or glutarimide ring.

#3665 Thrombospondin-1 mimetic peptides as inhibitors of angiogenesis. Fortuna Haviv, Michael Bradley, Douglas Kalvin, Andrew Schneider, Sandra Majest, Randy Bell, Donald Davidson, Catherine Haskell, Kennan Marsh, Bach Nguyen, Rae D. Record, Jason Hodde, Stephen F. Badylak, Yi-Chun Wang, David Frost, and Jack Henkin. *Abbott Laboratories, Abbott Park, IL, and Purdue University, West Lafayette, IN.*

Thrombospondin-1 (TSP-1) is a naturally occurring inhibitor of angiogenesis that has been shown to block many of the functions of activated endothelial cells (EC) and to mitigate tumor growth and metastasis. The large size, the scarcity and the multiple biological activities of TSP-1 make it impractical for direct use in cancer therapy. The peptide, NAc-Gly-Val-Dlle-Thr-Arg-Ile-ArgNHEt, a modified fragment of TSP-1, was reported to inhibit EC migration *in vitro* (D. Dawson et al, Mol. Pharmacol., 55: 332-338, 1999). However, because of its short half-life in rodents this heptapeptide was not suitable for therapeutic use. To reduce its basicity and the likelihood of histamine release, we substituted Nva in place of the internal Arg. To increase metabolic stability we substituted Pro at the C- and Sar at the N-termini resulting in ABT-526, NAc-Sar-Gly-Val-Dlle-Thr-Nva-Ile-Arg-ProNHEt. ABT-526 inhibited VEGF stimulated HMVEC migration with an IC_{50} of 0.03 nM, abrogated EC tube formation in fibrin gels at concentrations from 100-200 nM and inhibited neovascularization in a mouse cornea angiogenesis model when delivered by sc or ip injection. Replacement of Dlle in ABT-526 with Dallolle provided ABT-510, which displayed similar antiangiogenic activity *in vitro* and also inhibited bFGF-induced neovascularization in a mouse cornea model when delivered continuously at 30 mg/kg/day. ABT-510 had increased water solubility and slower clearance in primates, compared with ABT-526. In summary ABT-526 and ABT-510 are two examples of a series of small peptide TSP-1 mimetics that inhibit angiogenesis *in vitro* and *in vivo* and have pharmacokinetic profiles potentially suitable for therapeutic use. ABT-510 is currently in Phase I clinical development.

#3666 Semisynthetic sulfaminoheparosansulfates from *E. coli* K5 polisaccharide are potent antitumor agents in B16-BL6 melanoma. Andreina Poggi, Cosmo Rossi, Anna Maria Naggi, Luisa Sturiale, and Benito Casu. *Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy, and Istituto Scientifico G. Ronzoni, Milano, Italy.*

Heparin and related glycosaminoglycans can inhibit cancer cell invasion, due to their pleiotropic effects. The aim of our study was: 1) to evaluate the antitumor and anticoagulant activities of a series of semysinthetic sulfaminoheparosansulfates (SAHS) obtained by chemical modifications of Kappa 5 polysaccharide of *Escherichia coli*, in comparison with heparin; 2) to better understand the role of chemical sulfation, in relation to antitumor activity.B16-BL6 melanoma cells (100.000 cells/mouse) were injected intravenously in C57BL6 mice, with or without SAHS, at the dose of 0.5 mg/mouse. The number of tumor lung nodules was significantly inhibited, as compared to controls, only by heparin (95.5 percent), SAHS-2 (84.2 percent) and SAHS-4 (91.1 percent), belonging to type B compounds, characterized by selective distribution of sulfated groups at 2-O or 3-O position of glucuronic acid residues. SAHS-4 showed a dose-dependent inhibition (IC-50 0.1 mg/mouse) similar to heparin (0.05 mg/mouse). The anticoagulant potency, measured by activated partial thromboplastin time (APTT) on mouse plasma, indicated a better and more prolonged effect of heparin, in comparison with SAHS-4. Accordingly, H but not SAHS-4 significantly inhibited B16BL6 lung colonies, given 1 h before tumor cells. SAHS-4 derived compounds with higher or lower molecular weight or affinity depleted of AT-III binding site were as active as the original compound, suggesting that the antitumor activity was not dependent on AT-III mediated inhibition of blood clotting. Interactions with other blood clotting inhibitors cannot be ruled out. The better activity of heparin may be due to a longer presence in the circulation and/or to a better ability to inhibit neoangiogenesis.

#3667 Structure-based design of bifunctional S1-site inhibitors of human urokinase-type plasminogen activator. Lorraine M. Deck, Justin Heynekamp, Louis E. Metzger IV, William Brown, M. Brown, Vince N. Montes, and David L. Vander Jagt. *University of New Mexico School of Medicine, Albuquerque, NM.*

The urokinase-type plasminogen activator (uPA) system consisting of the serine protease uPA, its receptor uPAR, and associated proteins plays a major role in metastasis through directional activation of plasminogen and other proteins required for degradation of extracellular matrix. High levels of uPA and uPAR in a number of cancers predict poor outcome. Both uPA and uPAR are attractive candidates for development of anti-metastatic drugs. In previous studies of serine esterases, we demonstrated that substituted 6-chloro-2-pyrones represented a class of active-site directed inhibitors which could be potent and selective. Molecular modeling studies indicated that these inhibitors would also bind to the active site of uPA which was confirmed experimentally. Since uPA demonstrates specificity for a specific arginine-X bond, we have taken a structure-based approach to the design of uPA inhibitors that include an arginino mimetic attached to the pyrone backbone. Modeling studies predict that these inhibitors are bifunctional active site inhibitors in which both the pyrone moiety and the arginino mimetic moiety will occupy the S1 subsite of uPA. In addition, these inhibitors have the potential to function as suicide substrates of uPA.

#3668 Synthesis of dihydropyridine derivates of genistein to inhibit glioblastoma invasion. Launa M. J. Lynch, Adeboye Adejare, and Alok Bhushan. *Idaho State University, Department of Pharmaceutical Sciences, College of Pharmacy, Pocatello, ID.*

Glioblastoma represents the major form of primary brain tumors. The growth of the tumor and its invasive character are responsible for poor patient prognosis. Previous studies have shown that genistein blocks glioblastoma invasion, by inhibiting PLCγ-1 activation. The limitation of treating glioblastoma with genistein as a therapeutic agent could be due to its low distribution in the brain. To overcome this synthesize of dihydropyridine derivatives of isoflavones has been proposed. Based on a previous report dihydropyridine derivatives have been